Response surface optimization of the cacao criollo fermentation process in the province of Utcubamba, Amazonas-Peru

Optimización mediante superficie de respuesta del proceso de fermentación del cacao criollo en la provincia de Utcubamba, Amazonas-Perú

Optimização através da superfície de resposta do processo de fermentação do cacau criollo na província de Utcubamba, Amazonas-Peru

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Abstract

The fermentation process of native fine aroma cacao criollo (*Theobroma cacao*) in the province of Utcubamba, Amazonas, Peru, was optimized. A Box-Behnken statistical design was applied, whose factors were inoculum concentration, fermentation time and inoculation sequence. With the optimized model, a phenolic content of 12.99 mg AGE.g^{-1} cocoa, a fermentation index of 1.05, and theobromine and caffeine contents of 4.89.100 g^{-1} cocoa and 1.81.100g^{-1} cocoa, respectively, were obtained. Additionally, with a panel of nine certified and accredited tasters, the basic and special descriptive qualitative sensory attributes of the fermented and dry cocoa beans were determined, obtaining a maximum quality score of 71.1, and the sensory descriptors floral, fruity, nutty, sweet, bitter, acidity and adequate astringency were identified. In the cocoa obtained with the best treatment, 64 volatile compounds from the families of aldehydes, ketones, alcohols, esters, acids and pyrazines were identified by gas chromatography coupled to a mass detector with solid-phase microextraction (GC-MS-SPME-HS). In conclusion, it was possible to optimize the fermentation process of cacao criollo to obtain cocoa with high functional and sensory properties.

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Resumen

Se optimizó el proceso de fermentación de cacao (*Theobroma cacao*) criollo nativo fino de aroma en la provincia de Utcubamba, Amazonas, Perú. Se aplicó un diseño estadístico de Box-Behnken cuyos factores fueron la concentración de inóculo, tiempo de fermentación y secuencia de inoculación. Con el modelo optimizado se obtuvo un contenido fenólico de 12,99 mg AGE.g^{-1} cacao, un índice de fermentación de 1,05, contenidos de teobromina y cafeína de 4,89.100 g^{-1} cacao y 1,81.100g^{-1} cacao, respectivamente. Adicionalmente, con un panel de nueve catadores certificados y acreditados, se determinaron los atributos sensoriales básicos y especiales descriptivos cualitativos del cacao en grano fermentado y seco, obteniendo una puntuación de calidad máxima de 71,1 y se identificaron los descriptores sensoriales, floral, frutal, nuez, dulce, amargo, acidez y astringencia adecuada. En el cacao obtenido con el mejor tratamiento, se identificaron 64 compuestos volátiles de las familias de aldehídos, cetonas, alcoholes, ésteres, ácidos y pirazinas, mediante cromatografía de gases acoplada a un detector de masas con micro extracción en fase sólida (GC-MS-SPME-HS). En conclusión, se logró optimizar el proceso de fermentación de cacao criollo que permite obtener cacao con elevadas propiedades funcionales y sensoriales.

Palabras clave: Índice de fermentación, cacao nativo, cacao criollo, *Theobroma cacao*.

Resumo

O processo de fermentação do cacau criollo nativo de aroma fino (*Theobroma cacao*) na província de Utcubamba, Amazonas, Peru, foi otimizado. Um projeto estatístico Box-Behnken foi aplicado com concentração de inóculos, tempo de fermentação e seqüência de inoculação como fatores. Com o modelo otimizado, foi obtido um conteúdo fenólico de 12,99 mg de cacau AGE.g^{-1}, um índice de fermentação de 1,05, teobromina e cafeína de 4,89,100 g^{-1} de cacau e 1,81,100 g^{-1} de cacau, respectivamente. Além disso, com um painel de nove provadores certificados e credenciados, foram determinados os atributos sensoriais básicos e os atributos descritivos qualitativos especiais das amêndoas de cacau fermentadas e secas, obtendo-se uma pontuação máxima de qualidade de 71,1 e foram identificados os descritores sensoriais florais, frutados, frutos secos, doces, amargos, acidez e adstringência. No cacau obtido com o melhor tratamento, 64 compostos voláteis das famílias de aldeídos, cetonas, álcoois, ésteres, ácidos e pirazinas foram identificados por cromatografia gasosa acoplada a um detector de massa com microextração de fase sólida (GC-MS-SPME-HS). Em conclusão, foi possível otimizar o processo de fermentação do cacau criollo para obter cacau com altas propriedades funcionais e sensoriais.

Palavras-chave: Índice de fermentação, cacau nativo, cacau criollo, *Theobroma cacao*.

Introduction

Among the different processes that cocoa beans must go through (*Theobroma cacao*), fermentation is the first step in the chocolate chain (De Melo *et al*., 2013) this process is important and beneficial for the development of sensory characteristics. Microbial activity in cocoa beans removes the mucilage and induces a set of internal biochemical reactions in the cotyledon, which modify the chemical composition of the bean and initiate the formation of aroma precursors. During fermentation, microbial succession is produced by the variation of temperature, pH, oxygen availability and the compounds generated (Kongor *et al*., 2016), which makes the results very heterogeneous, so the optimization of this process will enhance and preserve the bromatological and organoleptic characteristics of cocoa that guarantees a good quality and homogeneous product (Sandhya *et al*., 2016).

According to Wacher (2011), yeasts and bacteria (lactic and acetic) are responsible for the fermentation of cocoa pulp, which contains carbohydrates (glucose, fructose and sucrose) at a pH between 3.3 and 4.0. They indicate that it is also necessary the presence of citric acid, appropriate medium for the development and proliferation of microorganisms that are essential for the transformation of cocoa beans; on the other hand, the production of acetic acid, ethanol and the increase of temperature, avoid the damage of the cocoa bean preventing the enzymatic action. .

The indicator of an optimal fermentation process is the ''fermentation index'' which must be equal to or higher than one (1) (León-Roque *et al*., 2016). In this sense, works have been carried out that have sought to improve this indicator using starter cultures that increase the efficiency of fermentation. In this regard, Cempaka *et al*. (2014), reported the increase of the fermentation index from 0.84 to 1.13 by initial addition of yeast, and Kresnowati *et al*. (2013) observed that by employing a starter culture composed by yeast and lactic acid bacteria, the fermentation index is improved from 0.86 to 0.95. Therefore, the objective of this work was to optimize the fermentation process of native fine aroma cacao criollo in the province of Utcubamba, Amazonas, Peru, by means of a response surface.

Materials and methods

The cocoa samples were acquired in slime from the La Cruz sector of the Cajaruro district, province of Utcubamba, Amazonas, Peru, a native (cacao criollo) producing area of the so-called fine aroma (common in northeastern Peru), characterized by an average altitude of 490 m above sea level, an average temperature of 29 °C and humidity of 61%.

The samples were transported in polypropylene bags to the facilities of the Cooperativa Central de Productores Agropecuarios de Amazonas (CEPROAA), where the fermentation experiment was carried out in cubic boxes of laurel wood (*Cordia alliodora*), 30 cm on each side, with 20 kg of cocoa in slime for each treatment. After the fermentation process (according to treatments), the fermented cocoa was dried in the sun until a moisture content of less than 7% was obtained, then it was packaged in airtight polyethylene bags and transferred to Laboratorio de Control de Calidad de Cacao of Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, for further analysis.

Obtaining the starter culture

The procedure described by Ausubel *et al*. (1989) was followed. For this purpose, pure cultures (yeasts + lactic acid bacteria [BAL] + acetic acid bacteria [BAA]) were isolated and biomass was obtained, achieving a frank development of yeasts in 250 mL flasks with liquid YPD culture medium prepared from meat peptone (20 g.L^{-1}), yeast extract (10 g.L^{-1}) and glucose/dextrose (20 g.L^{-1}) in ultra-pure water (Ausubel *et al*., 1989); the medium contained chloramphenicol for bacterial growth inhibition. For lactic and acetic acid bacteria, MRS

and Acetobacter broths were used. During fermentation, removal or turning was carried out every 24 h, until completing the programmed time according to the treatment.

Determination of the fermentation index (IF)

The IF was determined following the spectrophotometric method described by Gourieva & Tserevitinov (1979), given by the ratio of absorbances between 460 and 530 nm.

Determination of total phenol content

The determination of total phenols was carried out using the Folin Ciocalteu assay, for which, a standard calibration curve was prepared from dilutions of 0 to 16 $mg.L^{-1}$ of gallic acid (Sigma Aldrich, Germany), following the procedure described by Pantelidis *et al*. (2007). Zero point zero five milliliters (0.05 mL) of cocoa extract and 0.45 mL of water with 2.5 mL of Folin-Ciocalteu reagent (Merck, Germany) diluted 1:10, followed by two (2) mL of Na_2CO_3 at 7.5 % (m/v); then, they were completely mixed for 10 seconds in Vortex. It was incubated in an oven at 50 ºC for five (5) min, subsequently; the absorbance was measured at 765 nm in a spectrophotometer (UV-Visible S2100 UVTE, ÚNICO).

Determination of theobromine and caffeine

Theobromine and caffeine were determined by high performance liquid chromatography (HPLC) following the method described by Brunetto *et al*. (2007), using a chromatograph (Hitachi-Chromaster, Tokyo, Japan, (LC-20AD), equipped with an autoinjector (SIL-20A/HT), a communication module (CBM-20A) and a detector with photodiode array (PDA, SPD-M20A), ultraviolet detection was recorded at 278 nm. The separation was carried out on a 5 µm Supelco-LiChrospher RC C-18 column (25 cm x 4.6 mm). A methanol/water mixture (30:70 v/v) was used as mobile phase in isocratic mode at a flow rate of 1.0 $mL.min^{-1}$. The identification of the signals was performed by comparing the retention times obtained with theobromine and caffeine standards (98 % Sigma-Aldrich, USA). For the quantification of the concentrations of theobromine and caffeine in the cocoa samples, a calibration curve was prepared for theobromine and caffeine in a range from 5 to 100 $mg.L^{-1}$, with whose areas under the curve the respective linear function was obtained.

Determination of the pH of cocoa beans

The pH of the samples was measured following the methodology described by Senanayake *et al*. (1999).

Preparation of samples for sensory analysis and volatile compounds

The whole, fermented and dried beans obtained according to the treatments were roasted in a laboratory roaster (IMSA, Peru) of one (1) kg capacity at 120 °C for 15 min. Then, they proceeded to shelling in a sheller (IMSA, Peru). The nibs obtained were subjected to a conching process for 4 h at 50 °C, molded into 50 g tablets, packed in aluminum foil and polyethylene bag with hermetic seal. The tablets were stored frozen until further analysis.

Sensory analysis of cocoa pastes

The fermented cocoa according to treatment was dried and processed into paste. Then, with nine (9) judges certified and accredited by APPCacao-Sineace-Minedu-Peru, the basic and special sensory attributes (cocoa flavor, floral, fruity, nutty, sweet, bitter, acidity and astringency) were evaluated with the ordinal scale from 1 to 100 for the total score, given by the sum of the partial scores of each attribute, using the Tasting Card for the sensory analysis of cocoa (USAID, Equal Exchange and TCHO, 2018).

Identification of the volatile compounds in cocoa paste

Volatile compounds were identified using gas chromatography coupled to a mass detector and headspace-micro solid-phase extraction (GC-MS-SPME-HS) (Rodriguez-Campos *et al*., 2012), employing a divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS), 50/30 µm thick and helium as carrier gas. In 20 mL vials, 5.7 g of cocoa paste was placed, six (6) mL of ultrapure water was added and hermetically sealed with a metallic lid and a 20 mm white silicone septa. The SPME extraction conditions were: 15 minutes of equilibrium at 50 °C, with an exposure of the fiber for 30 min at the same temperature. After the extraction time, the fiber was retracted and immediately inserted into the injection port of the gas chromatograph-mass spectrometer (GC-MS) (Angilent technologies, 6890N, United States), where it was kept for five (5) min at 250 °C temperature. Between each extraction a run of the fiber blank (clean-up) was performed for 55 min. For the identification of the compounds, the NIST 14.L library was used.

Experimental design and statistical analysis

The Box-Behnken statistical design (Murali *et al*., 2000) was used to determine the best concentration values of inoculum X1 (1.0; 1.8 and 2.6 x 10^3 $UFC.mL^{-1}$), inoculation sequence X2 (yeasts + BAL + BAA at the beginning; yeasts and BAL at the beginning+ BAA at 48 h; yeasts at the beginning + BAL at 24 h + BAA at 48 h), and fermentation time X3 (5, 6 and 7 days), as shown in table 1. The responses of interest were the fermentation index (IF), chemical characteristics (total phenols, theobromine, caffeine and pH), sensory characteristics and volatile compounds.

Table 1. Box-Behnken arrangement for optimization.

Number	Variables		
Experiment	X1	X2	X3
1	0	-	-
2	+	-	0
3	0	0	0
4	0	-	+
5	-	0	+
6	0	+	+
7	-	0	-
8	-	-	0
9	0	0	0
10	0	0	0
11	+	+	0
12	-	+	0
13	0	+	-
14	+	0	+
15	+	0	-

+: Upper level of the variables, 0: Average level of the variable. -: Lower level of the variables, X1: Inoculum concentration. X2: sequence of inoculation, X3: days of fermentation

Cocoa beans in slime were placed in the fermenters and 10 mL of the microorganisms were inoculated according to the treatments described. To obtain the optimum fermentation parameters with cocoa starter culture, under the Box Behnken design. A response surface analysis was performed using Minitab 19 software. The Kruskal Wallis non-parametric test was also applied to compare treatments according to sensory qualification.

Results and discussion

Table 2 shows the average values of chemical properties of fermented cocoa beans, in which a variation is observed due to the set of reactions generated in the fermentation process, accentuating some characteristic flavors and aromas of the samples, as described by Castro-Alayo *et al*. (2019).

Optimization of fermentation parameters

Table 3 shows the optimized values, obtaining an IF higher than 1, pH of 5.32, indicating an adequate fermentation, free of undesirable compounds or acids in the cocoa beans.

Table 2. Chemical properties of fermented cocoa.

Treatments	Total phenols (mg GAE.g^{-1})	Fermentation index	pH	Theobromine (g.100 g^{-1} cocoa)	Caffeine (g.100 g^{-1} cocoa)
T1	15.07±0.39	0.68±0.10	5.85±0.10	5.04	2.47
T2	15.57±0.11	0.71±0.08	5.82±0.05	4.93	2.31
T3	15.46±0.42	0.90±0.42	5.78±0.10	4.58	2.04
T4	13.78±0.32	0.95±0.04	5.65±0.10	5.07	2.23
T5	20.41±0.66	0.55±0.28	5.69±0.11	5.13	2.15
T6	15.23±0.21	0.78±0.11	5.86±0.10	5.06	2.06
T7	16.93±0.17	0.99±0.09	5.59±0.03	5.12	2.25
T8	16.73±0.27	0.99±0.09	5.63±0.02	5.65	2.68
T9	15.46±0.42	0.90±0.19	5.74±0.09	4.96	2.48
T10	15.46±0.42	0.90±0.19	5.70±0.09	4.92	1.92
T11	13.93±0.86	0.97±0.02	5.80±0.04	5.81	2.35
T12	12.17±0.17	1.25±0.19	5.17±0.05	5.00	2.05
T13	17.28±0.48	0.87±0.19	5.33±0.09	6.14	2.46
T14	12.90±0.66	1.05±0.15	5.32±0.06	4.98	1.78
T15	15.37±0.41	1.18±0.09	5.27±0.10	5.65	2.40

Table 3. Response and optimized values for chemical properties of the native fine aroma cocoa samples.

Response	Optimized	<95.0 %	>95.0 %
Total phenols (mg GAE.g^{-1})	12.99	7.80	18.19
Fermentation index	1.05	0.57	1.52
pH	5.32	4.97	5.67
Theobromine concentration (g.100 g^{-1} cocoa)	4.89	4.44	5.35
Caffeine concentration (g.100 g^{-1} cocoa)	1.81	1.37	2.25

The optimized model was determined: inoculum concentration 2.6 x 10^3 UFC.mL^{-1}, yeast inoculation sequence and BAL at the beginning + BAA at 48 h and fermentation time (7 days), to achieve an adequate fermentation process (figure 1).

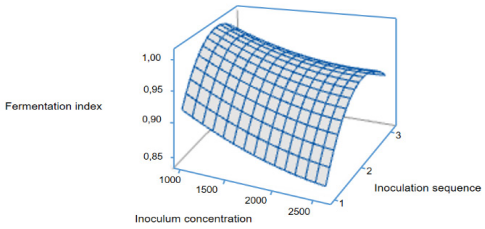

Figure 1. Response surface graph to optimize the fermentation index in the fermentation process of cacao criollo.

The optimized model (figure 2), allows to obtain fermented cocoa with low levels of total phenols (12.99 AGE.g^{-1}), therefore the pastes have low astringency (Ooi *et al*., 2020). Given the differences in the results, treatments 7, 8 and 11, due to their high fermentation index (table 2), improved microbial activity, they were able to hydrolyze free, soluble phenolic complexes, which are easily absorbed, leading to a decrease in seeds (Haile & Kang, 2019; Jalil & Ismail, 2008). This has been largely attributed to oxidation of insoluble tannins and leaching of almonds polyphenols into the surrounding pulp and subsequent runoff on sweating (Wollgast & Anklam, 2000).

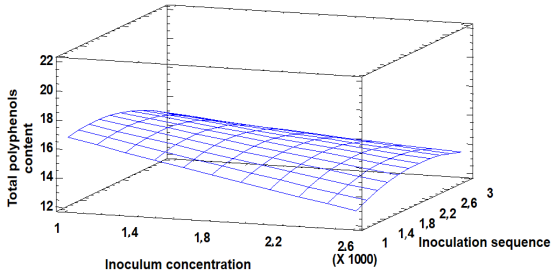

Figure 2. Response surface graph to optimize the phenolic content in the fermentation process of cacao criollo.

The application of the microorganisms allowed to obtain a fermentation index equal to 1.05 in seven (7) days, very consistent results due to the obtaining of completely fermented beans with well pronounced, light brown to dark brown striations (Ooi *et al*., 2020). It should be noted that the brown color formed is due to the oxidation reaction when catalyzing o-diphenol to o-quinone (Hernández-Hernández *et al*., 2016).

The model allowed to obtain an optimized pH value of 5.32; which indicates an adequate fermentation process, the pH of beans leads to the generation of more free peptides and amino acids, which would be able to form the Maillard reaction during roasting, contributing to more preferred aroma and flavor notes (Afoakwa *et al*., 2008; John *et al*., 2019).

Theobromine and caffeine concentrations

The optimal values of theobromine and caffeine were 4.89 and 1.81 g.100 g^{-1} cocoa respectively; values that indicate the intensity of bitterness in the cocoa paste (Brunetto *et al*., 2007), these compounds are associated with the index fermentation of cocoa beans (Cardoso *et al*., 2020).

Sensory analysis of native fine cacao criollo paste with fermented aroma with different treatments

Figure 3 shows the results obtained in the sensory analysis of the quality attribute of native fine aroma cocoa.

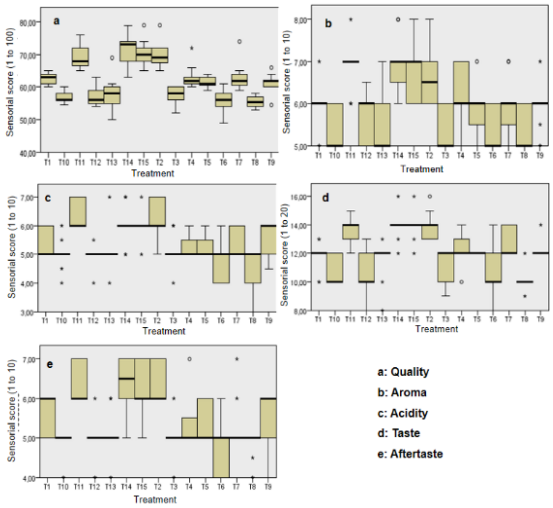

Figure 3. Sensory analysis of the quality attribute of native fine aroma cocoa.

Four treatments (T11, T14, T15 and T2) formed the best scored group (close to 70) in terms of quality of the cocoa paste obtained per treatment, as shown in figure 3a.

The same treatments that allowed obtaining higher quality were those that obtained the best aroma score, as shown in figure 3b. Average scores of seven (7) for treatments 11, 14 and 15, evidence that the fermentation was adequate and that the volatile compounds precursors of the aroma were enhanced. Fermentation treatments 11, 14, 15, 2 and 9, obtained the best scores in sensory acidity. The judges revealed a citric acidity, this could be due to 4-methylpentanoic and beta-myrcene acids, identified in T14, the treatment with the highest sensory acceptance ($p<0.05$), as can be seen in Figure 3c, which confer an acidity similar to a sweet orange, generated and transferred to the cotyledons during the fermentation process, being this type of acidity beneficial for special cocoa samples (Utrilla-Vázquez *et al*., 2020). The same treatments had the highest scores in flavor and aftertaste (figures 3d and 3e).

Identification of the volatile compounds of native fine aroma cocoa

The volatile compounds precursors of aroma were identified in treatment 14 (table 4), due to it was the best treatment. Among the identified volatile compounds, aldehydes, ketones, alcohols, esters, acids and pyrazines, mainly responsible for special aromas and flavors, such as Linalool, 2-Nonanol (floral, menthol, cinnamon, citrus and fruit aromas); 3-ethyl-2,5-dimethyl pyrazine, tetramethyl pyrazine, 2-ethyl-6-methyl pyrazine, trimethyl pyrazine, 2,3,5-trimethyl-6-ethylpyrazine (aromas of roasted cocoa, sweet panela, sweet caramel) (Utrilla-vázquez *et al*. , 2020). Figure 4 shows chromatogram of volatile compounds identified by GC-MS-SPME-HS.

Table 4. Volatile compounds identified in fermented samples of native fine aroma cacao criollo.

Compound name	Group	Chemical formula	TR	Compound aroma description*.
Dimethyl ether	Ether	C_2H_6O	4.855	Fruits
Acetone	Aldehydes and ketones	C_3H_6O	5.475	Fruits
2-Methylpropanal		C_4H_8O	7.096	Sweet
Acetic acid	Acid	$C_2H_4O_2$	7.625	Bitter, vinegar
2,3-Butanedione	Ketones	$C_4H_6O_2$	7.943	Buttery
2-Butanone		C_4H_8O	8.194	Sweet
Butanimidamide		$C_4H_{10}N_2$	10.688	…
2-Methylbutanal	Aldehydes and ketones	$C_5H_{10}O$	11.108	Malt, chocolate
2,3-Pentanedione	Ketones	$C_5H_8O_2$	12.345	Toasted almond, cocoa, yogurt, nuts
Pentanal	Aldehydes and ketones	$C_5H_{10}O$	12.541	Fruits
Acetoin	Aldehydes and ketones	$C_4H_8O_2$	12.982	Butter
3-(1methylethyl)oxoethane		$C_6H_{12}O$	14.283	...
2-Methylpropanoic acid	Acid	$C_4H_8O_2$	14.515	...
2,3-Butanediol	Alcohol	$C_4H_{10}O_2$	16.446	...
Cyclobutanol, 2-ethyl		$C_6H_{12}O$	17.664	…
Hexamethyl cyclotrisiloxane		$C_6H_{18}O_3Si_3$	18.061	…
3-Furaldehyde	Aldehydes and ketones	$C_5H_4O_2$	19.34	Toasted almond
Butanoic acid, 2-methyl	Acid	$C_5H_{10}O_2$	19.477	…
3-Furanmethanol	Alcohol	$C_5H_6O_2$	20.166	Mint
2-Heptanone	Aldehydes and ketones	$C_7H_{14}O$	22.094	Fruit, plantain
Styrene		C_8H_8	22.656	Sweet, cinnamon, coffee.
1-(2-Furanyl) ethanone		$C_6H_6O_2$	23.214	…

*(Utrilla-vázquez *et al*., 2020).

Table 4. Volatile compounds identified in fermented samples of native fine aroma cacao criollo (continuation).

Compound name	Group	Chemical formula	TR	Compound aroma description*.
2,5- Dimethylpyrazine	Pyrazines	$C_6H_8N_2$	23.401	Caramel
2,3- Dimethylpyrazine		$C_6H_8N_2$	23.708	Roasted cocoa
4-Methylpentanoic acid		$C_6H_{12}O_2$	23.950	Fruity, pineapple
2-Furancarboxaldehyde, 5-methyl		$C_6H_6O_2$	25.692	…
Octametil ciclotetrasiloxano		$C_8H_{24}O_4Si_4$	25.884	…
Benzaldehyde	Aldehydes and ketones	C_7H_6O	26.094	Pleasant almonds
Beta-Myrcene		$C_{10}H_{16}$	26.965	Citrus
Benzonitrile	Nitriles	C_7H_5N	27.061	Almonds
2-Ethyl-6-methylpyrazine,		$C_7H_{10}N_2$	27.435	Sweet caramel
Trimethylpyrazine	Pyrazines	$C_7H_{10}N_2$	27.617	Roasted cocoa
2-Ethenyl-6- methylpyrazine		$C_7H_8N_2$	28.385	Sweet caramel
Acetate 2-heptanol		$C_9H_{18}O_2$	28.845	…
3,6,6- trimethylbicyclo (3.1.1) hept-2-ene		$C_{10}H_{16}$	28.975	…
Benzyl alcohol	Alcohol	C_7H_8O	29.129	Sweet, flowery
1-Methyl-5-(1-methylethenyl) cyclohexene		$C_{10}H_{16}$	29.199	Essential oils, citrus
1,3,3-Trimethyl, tricyclo[2.2.1.0(2,6)]heptane		$C_{10}H_{16}$	29.509	Essential oils, citrus
Benzeneacetaldehyde	Aldehydes and ketones	C_8H_8O	29.738	Almond, cherry, strawberry
1-(1h-pyrrol-2-yl) ethanone,		C_6H_7NO	30.161	…
3-Methyl 2-cyclohexen-1-one		$C_7H_{10}O$	30.325	…
Acetophenone	Aldehydes and ketones	C_8H_8O	30.783	Sweet
3-Ethyl-2,5- dimethylpyrazine	Pyrazines	$C_8H_{12}N_2$	30.913	Roasted cocoa,
Tetramethylpyrazine		$C_8H_{12}N_2$	31.267	roasted, chocolate
2-Methoxyphenol		$C_7H_8O_2$	31.507	Sweet
Ethyl 2-(5-methyl-5-vinyl tetrahydrofuran-2-yl)propan -2-yl carbonate		$C_{13}H_{22}O_4$	31.575	…
2-Nonanol	Alcohol	$C_9H_{20}O$	31.715	Fruits, roses
Linalool		$C_{10}H_{18}O$	31.810	Floral, mentholated, cinnamon, citrus
Nonanal	Aldehyde	$C_9H_{18}O$	31.984	Rose, orange, citrus
Phenylethyl alcohol	Alcohol	$C_8H_{10}O$	32.718	Floral
2,3-Dimethyl 2,4,6-Octatriene		$C_{10}H_{16}$	33.007	Citrus, essential oils
4h-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		$C_6H_8O_4$	33.918	…
2,3,5- Trimethyl-6-ethylpyrazine	Pyrazines	$C_9H_{14}N_2$	34.204	Sweet caramel
Ethyl bezoate	Ester	$C_9H_{10}O_2$	35.026	Sweet, fruit, cherry, grapes.
(3R,6S)-2,2,6- Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	Alcohol	$C_{10}H_{18}O_2$	35.155	…
Octanoic acid, ethyl ester	Ester	$C_{10}H_{20}O_2$	35.468	Orange aroma, citrus
Dodecane		$C_{12}H2_6$	35.81	…
Benzeneacetic Acid Ethyl ester		$C_{10}H_{12}O_2$	37.666	…
Acetic acid, phenylethyl ester		$C_{10}H_{12}O_2$	38.167	…
Benzeneacetaldehyde, alpha.-ethylidene		$C_{10}H_{10}O$	38.957	Green, fresh

*(Utrilla-vázquez *et al*., 2020).

Table 4. Volatile compounds identified in fermented samples of native fine aroma cacao criollo (continuation).

Compound name	Group	Chemical formula	TR	Compound aroma description*.
Cyclotetradecane		$C_{14}H2_8$	42.865	...
1-Butanol, 3-methyl-, benzoate		$C_{12}H_{16}O_2$	43.289	…
5-Methyl-2-phenyl-2-hexenal		$C_{13}H_{16}O$	46.478	Cocoa
1H-2-Benzopyran-1-one, 3,4-dihydro- 8-hydroxy-3-methyl		$C_{10}H_{10}O_3$	48.988	…

*(Utrilla-vázquez *et al*., 2020).

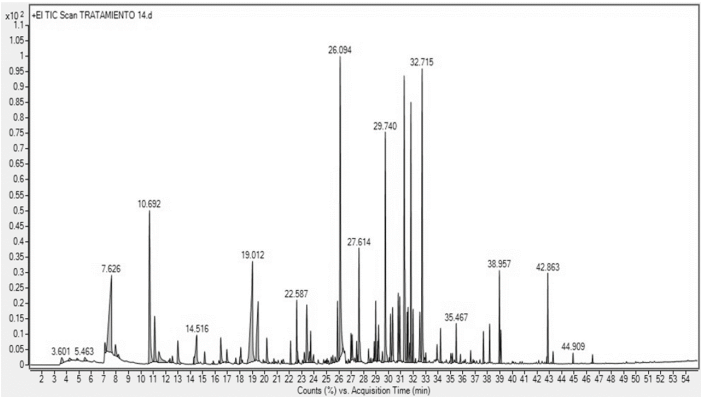

Figure 4. Chromatogram of volatile organic compounds precursors of the aroma of the T14 treatment.

Sixty-four volatile compounds were identified: 14 aldehydes and ketones; 7 pyrazines, 7 alcoholic compounds, 4 esters, 4 organic acids and other compounds, which confer the special notes to the cocoa samples (Rodríguez-Campos *et al*., 2012). At the end of fermentation, aldehydes and ketones represent the highest percentage of the total content of volatile compounds, which confer the pleasant notes of almond, butter or floral; pyrazines and alcohols are among the most important flavor groups of cocoa as they confer notes of chocolate, roasted coffee, fruity, floral, menthol, cinnamon, citrus and especially the Linalool volatile compound present in samples of cocoa from the Amazon region. Similar compounds were found in samples of Criollo and Trinitario cocoa grown in Chiapas, Mexico (Utrilla-Vázquez *et al*., 2020).

Conclusions

This study allowed to optimize the fermentation process of *T. cacao* native fine aroma, finding the optimal model. Emphasizing the importance of the fermentation process presented in the particular characteristics of the chemical composition and volatile compounds of native fine aroma cocoa from the Amazon region.

The microorganisms added in the fermentation process allowed accelerating a set of chemical reactions that give rise to the formation of flavor precursors and the formation of a qualitatively and quantitatively very important aroma precursor fraction, which was evidenced by the volatile compounds identified.

Regarding to the sensory analysis, a final maximum quality score of 71.1 was obtained, influencing the astringency due to the concentration of phenols, the bitterness due to the theobromine and caffeine content, and the special flavors and aromas due to the volatile compounds, especially from the aldehyde, ketone, ester, pyrazine and alcohol families, which confer these special notes to cocoa, allowing to have extraordinary samples.

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